

Figure 1 Inhibition of labelled TSH binding to TSHR coated tubes by hMAb TSHR1 IgG and Fab. The control IgG was a human monoclonal autoantibody to GAD₆₅.

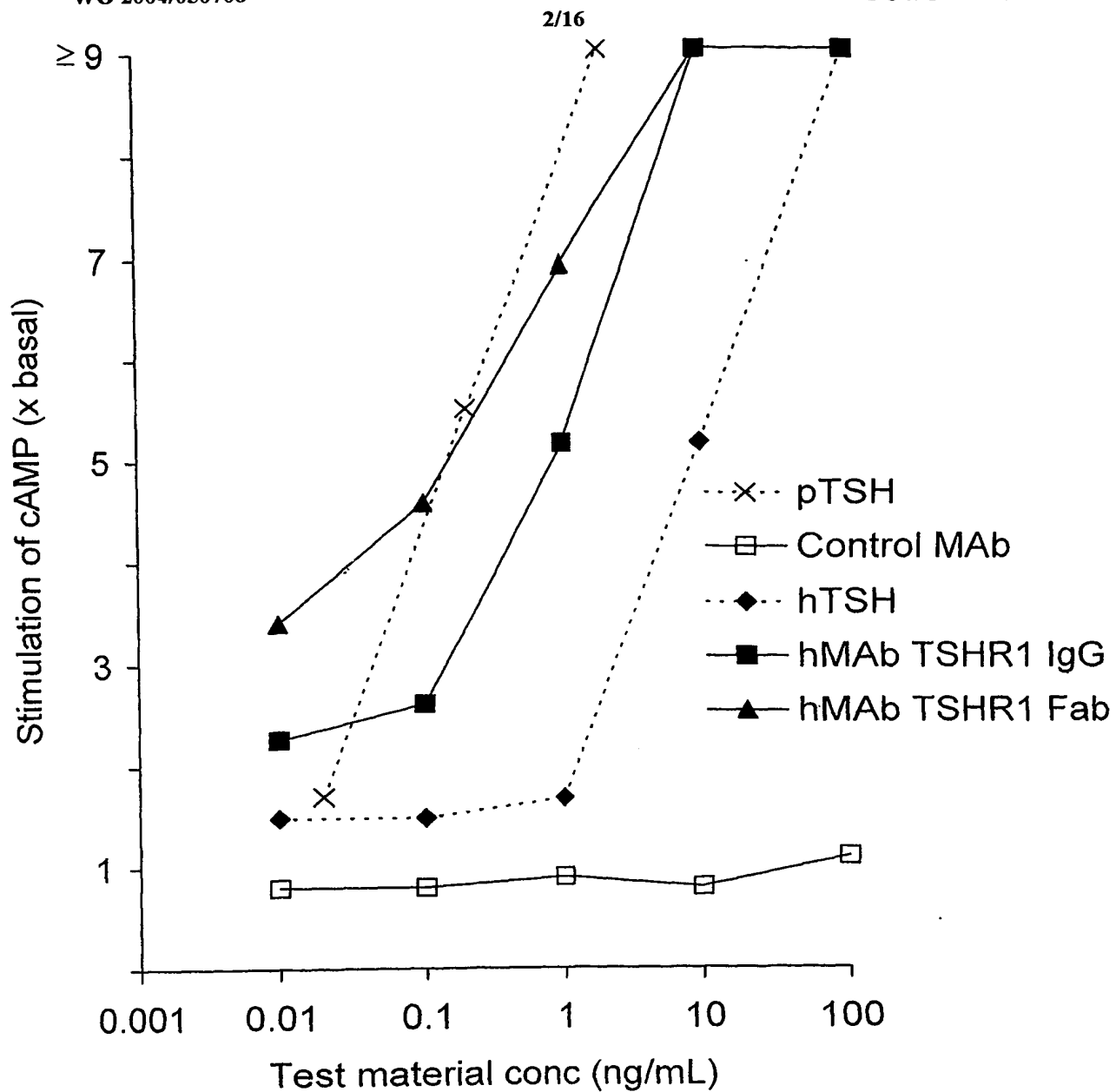


Figure 2 Thyroid stimulating activities of hMAb TSHR1 IgG and Fab, porcine TSH (70 units/mg; pTSH), recombinant human TSH (6.7 units/mg; hTSH) and a control monoclonal antibody (MAb: a human monoclonal autoantibody to thyroid peroxidase (2G4)). Basal = cAMP produced in the presence of NaCl free Hanks Buffered Salt Solution only.

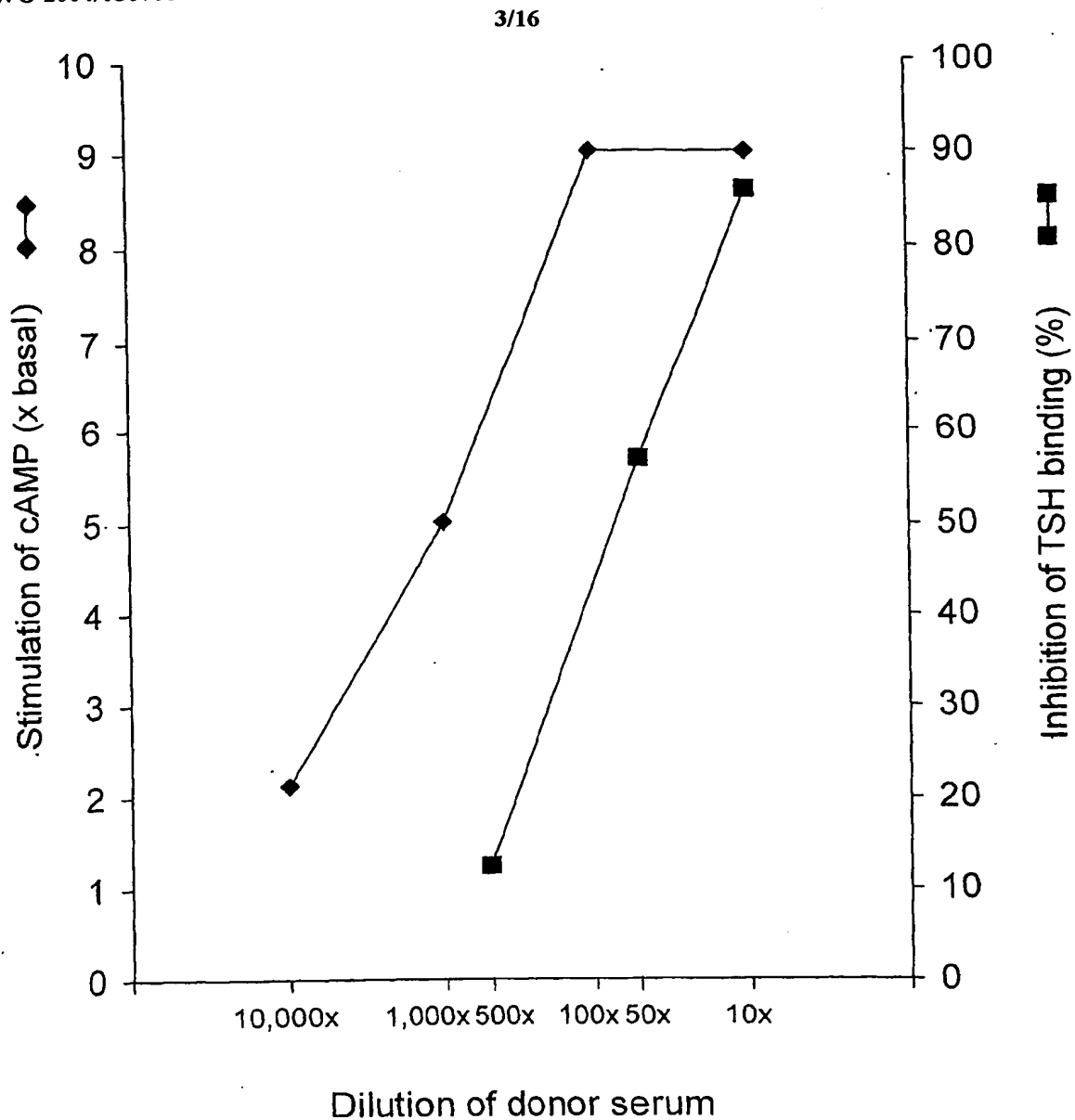
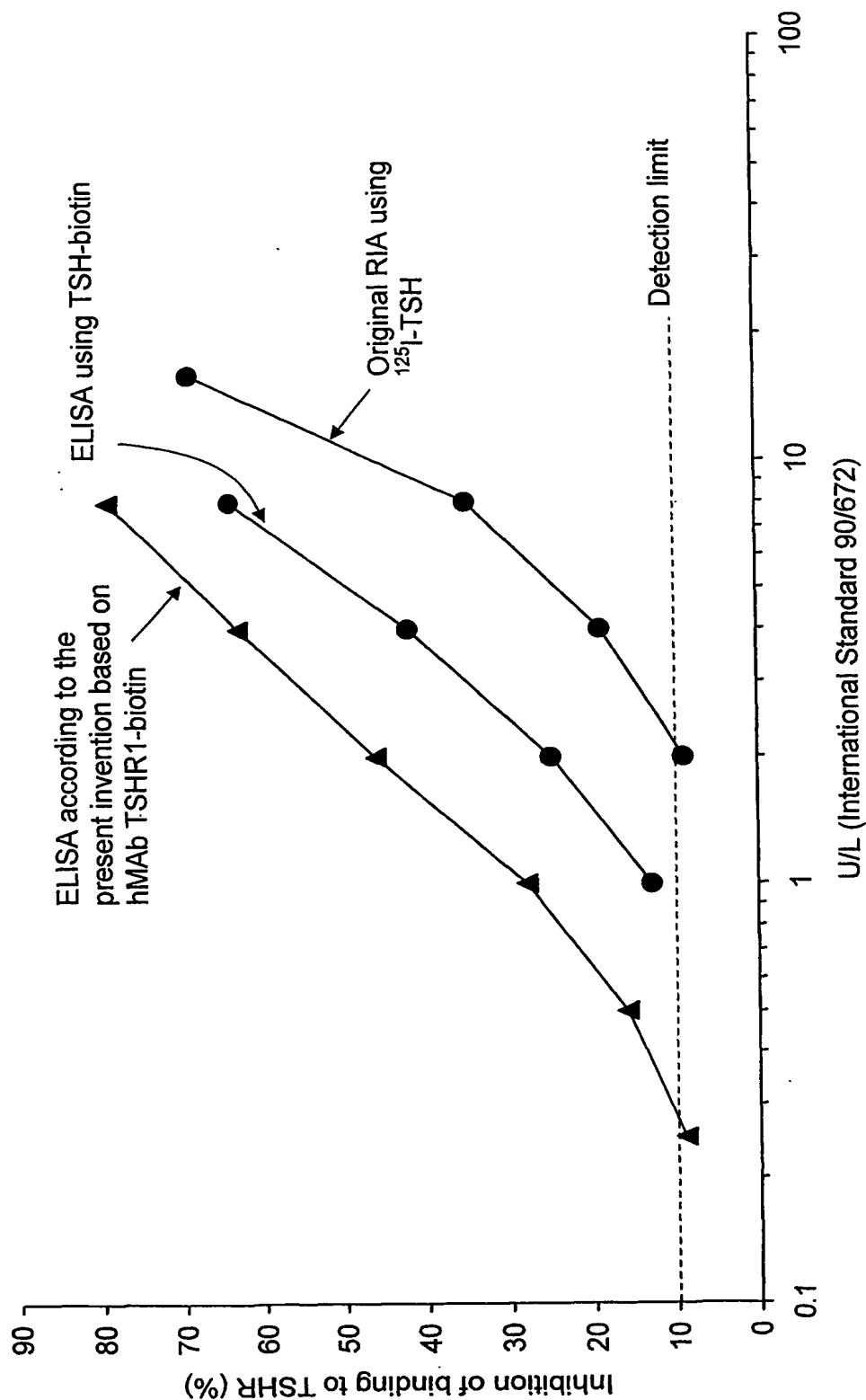


Figure 3 Effect of lymphocyte donor serum on inhibition of TSH binding to the TSHR and on stimulation of cyclic AMP in TSHR transfected CHO cells. In the case of the binding inhibition assay the serum was diluted in a pool of healthy blood donor sera. For the stimulation assay, the serum was diluted in NaCl free Hanks Buffered Salt Solution. Healthy blood donor sera ($n = 3$) gave responses ranging from 1.1 – 1.3 x basal.

Comparison of an ELISA for TSHR autoantibodies according to the present invention with earlier assays. In particular an ELISA based on TSH-biotin described by J Bolton, J Sanders, Y Oda, C Chapman, R Konno, J Furmaniak, B Rees Smith. "Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA." Clinical Chemistry 1999 volume 45 pp 2285-2287 and the original RIA described by K Southgate, FM Creagh, M Teece, C Kingswood, B Rees Smith. "A receptor assay for the measurement of TSH receptor antibodies in unextracted serum" 1984. Clinical Endocrinology volume 20 pp 539-543.

Figure 3a



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Figure 3b Comparison of an ELISA for TSHR autoantibodies according to the present invention and an ELISA based on TSH-biotin described by J Bolton, J Sanders, Y Oda, C Chapman, R Konno, J Furmaniak, B Rees Smith. "Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA." Clinical Chemistry 1999 volume 45 pp 2285-2287. Sera from 72 patients with Graves' disease were compared. $y = 1.1154x - 13.032$, $r = 0.99$.

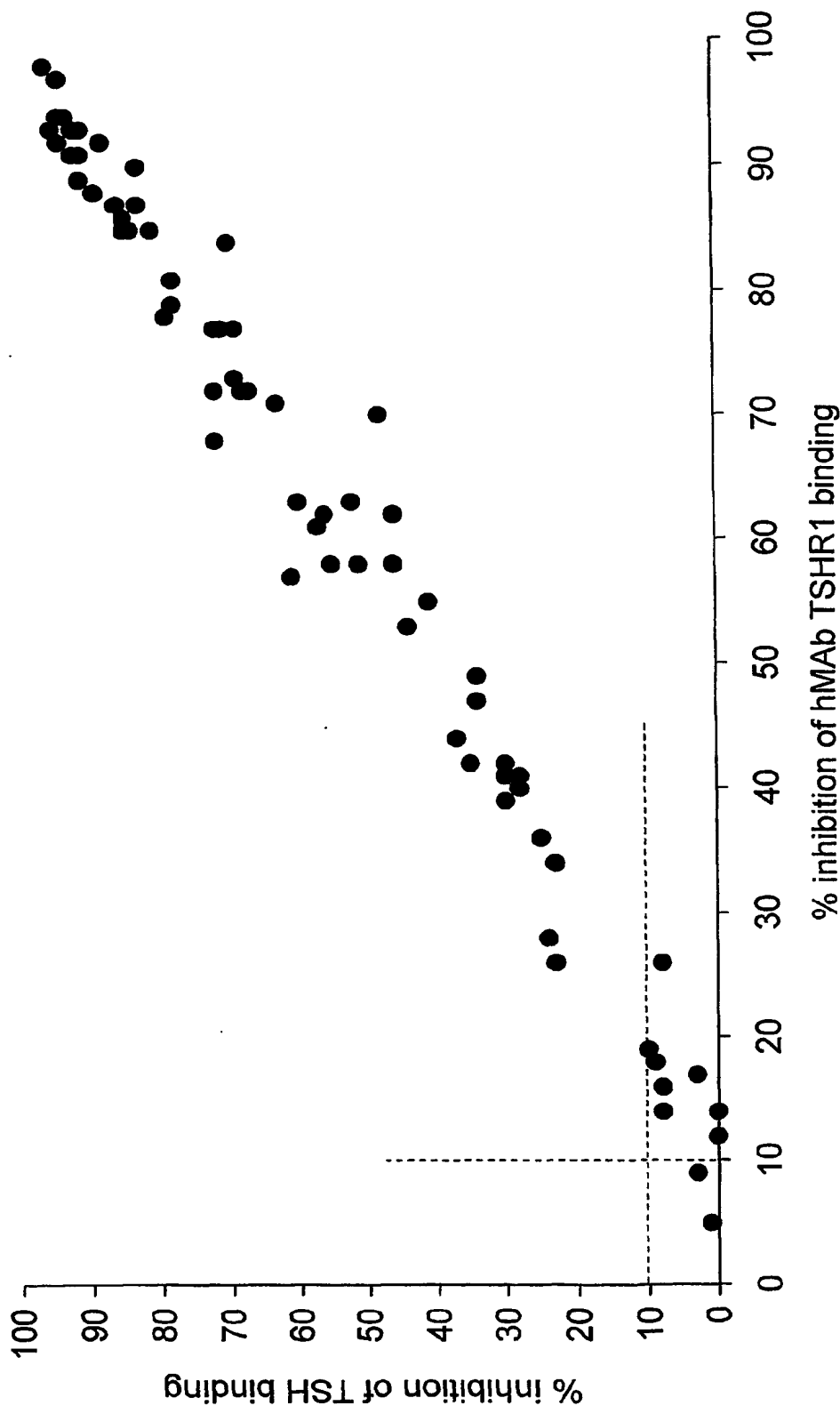


Figure 4 hMAb TSHR1 Heavy Chain V, D and J region nucleotide sequence**Figure 4a**

caaatgcagctggtgcagctctggagcagaggtgaaaaagcccggggagtc
tctgaagatctcctgtaggggttctggatacaggtttaccagctactgga
tcaactgggtgcgccagctgcccgggaaaggcctagagtggatgggcagg
attgatcctactgactcttataccaactacagtccatccttcaaaggcca
cgtcaccgtctcagctgacaagtccatcaacactgcctacctgcagtgga
gcagcctgaaggcctcggacaccggcatgtattactgtgcgaggctcgaa
ccgggctatagcagcacctgggtccgtaaattggggccagggaaccctggt
caccgtctcctcagcctccaccaagggcccatcggtcttcccc

Figure 4b

caaatgcagctggtgcagctctggagcagaggtgaaaaagcccggggagtc	50
PCR primer	
tctgaagatctcctgtaggggttctggatacaggtttaccagctactgga	100
CDR I	
teaatggggtgcgccagctgcccggaagggcctagagtggatgggagg	150
CDR II	
attgatcctaetgactcttataccaatcacagtcacatccttcaaaggcca	200
cgtcaccgtctcagctgacaagtccatcaacactgcctacctgcagtggg	250
gcagcctgaaggcctcggacaccggcatgtattactgtgcgaggctggaa	300
CDR III	
cgggctatagcagcacctgggtccgtaaattggggccagggaaccctggt	350
constant region	
caccgtctcctcagcctccaccaaggggcccatcggtcttccccc	394

Figure 5 hMAb TSHR1 Heavy Chain V, D and J region amino acid sequence

Figure 5a

QVQLVQSGAEVKKPGESLKISCRGSGYRFTSYWINWVRQLPGKGLEWMGR

IDPTDSYTNYSFSGHVTVSADKSINTAYLQWSSLKASDTGMYTCARLE

PGYSSTWSVNWGQGLTVTVSSASTKGPSVFP

Figure 5b

QVQLVQSGAEVKKPGESLKISCRGSGYRFTSYWINWVRQLPGKGLEWMGR	50
CDR I	
IDPTDSYTNYSFSGHVTVSADKSINTAYLQWSSLKASDTGMYTCARLE	100
CDR II	
PGYSSTWSVNWGQGLTVTVSSASTKGPSVFP	131
CDR III	
constant region	

Figure 6 hMAb TSHR1 Light Chain DNA sequence**Figure 6a**

ctgcctgtgctgactcagccaccctcggtgtctggagccccaggcagag
 ggtcaccatctcctgttctggaaacagctccaacatcggaataatgctg
 taaactggtaccagcagctcccaggaaaggctcccaaactcctcatttat
 tatgatgatcaactgccctcaggggtctctgaccgattctctggctccag
 gtctggcacctccgcctccctggccatccgtgggctccagtctgaggatg
 aggctgattattactgtacatcatgggatgacagcctggatagtcaactg
 ttcggcggaggaggaccaggctgaccgtcctaggt

Figure 6b

ctgcctgtgctgactcagccaccctcggtgtctggagccccaggcagag	50
PCR primer	
ggtcaccatctcctgttctggaaacagctccaacatcggaataatgctg	100
CDR I	
taaactggtaccagcagctcccaggaaaggctcccaaactcctcatttat	150
tatgatgatcaactgccctcaggggtctctgaccgattctctggctccag	200
CDR II	
gtctggcacctccgcctccctggccatccgtgggctccagtctgaggatg	250
aggctgattattactgtacatcatgggatgacagcctggatagtcaactg	300
CDR III	
ttcggcggaggaggaccaggctgaccgtcctaggt	333

Figure 7 hMAb TSHR1 Light Chain protein sequence

Figure 7a

LTVLTQPPSVSGAPRQRVTISCSGNSSNIGNNAVNWYQQLPGKAPKLLIY

YDDQLPSGVSDRFGSRSGTSASLAIRGLQSEDEADYYCTSWDDSLDSQL

FGGGTRLTVLG

Figure 7b

LTVLTQPPSVSGAPRQRVTISCS	SGNSSNIGNNAVNWYQQLPGKAPKLLIY	50
CDR I		
YDDQLPS	GVSDRFGSRSGTSASLAIRGLQSEDEADYYCTSWDDSLDSQL	100
CDR II	CDR III	
FGGGTRLTVLG		111

Figure 8 Effects of 2 patient sera (T1 and T2 with TSH antagonist activity) on stimulation of cyclic AMP production (in CHO cells transfected with the TSHR) by pTSH (0.5 ng/mL) and hMAb TSHR1 IgG (10 ng/mL) and Fab (5 ng/mL)

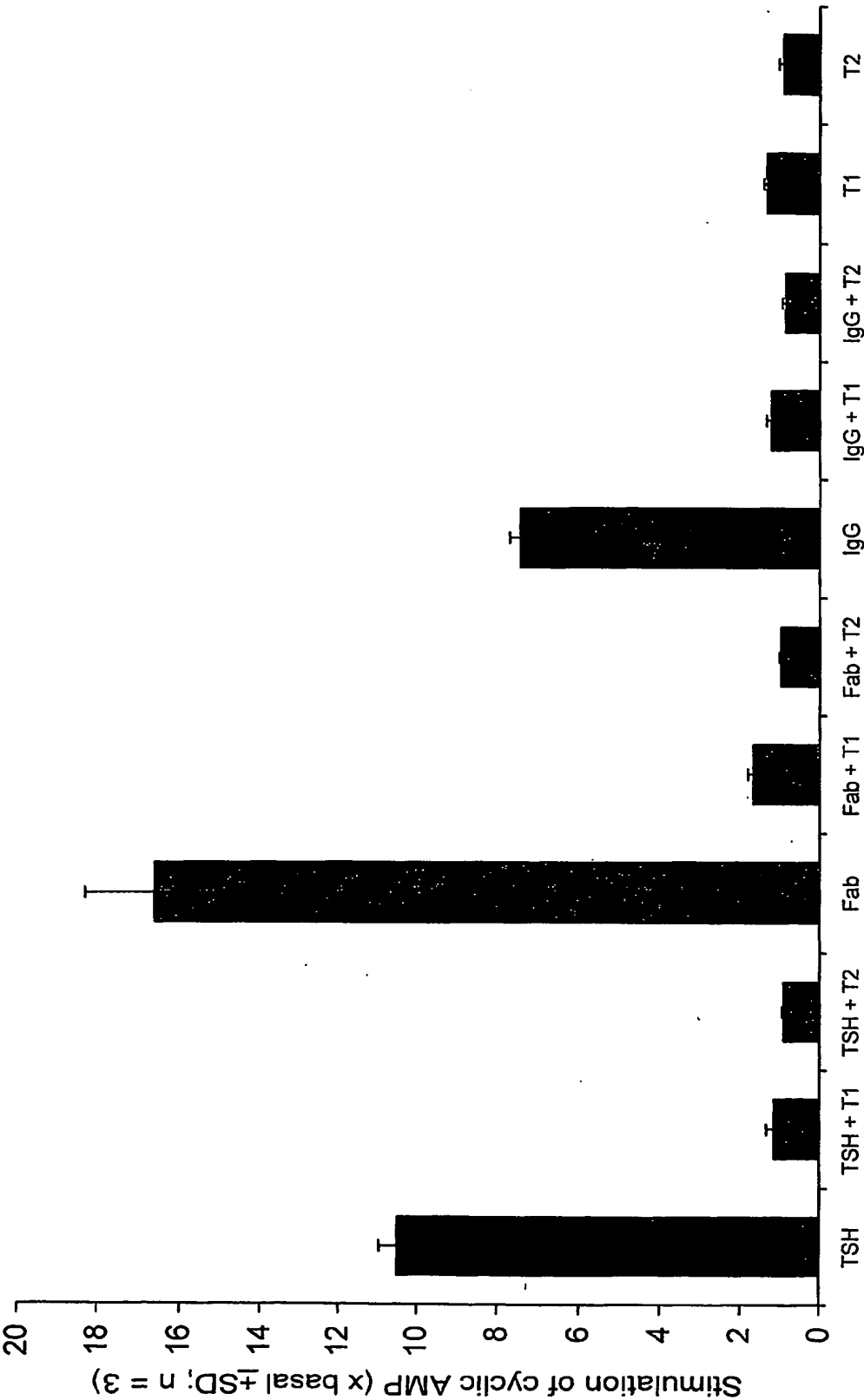


Figure 9 9D33 Heavy Chain nucleotide sequence

Figure 9a

gacgtccagatccagcagcctgggactgagcttgtgaagcctggggcttc
agtgagactgtcctgcaaggcttctggctacaccttcaccacctactgga
tgactgggtgaagcagaggcctggacaaggccttgagtggatcggagag
attgatccttctgatagttataactaactataatcaaaagttcaagggcaa
ggccacattgactgtagacaaatcctccagcacagcctacatgcacctca
gcagcctgacatctgaggactctgcggtctattactgttcaagaaactac
ggtagtggctactactttgactactggggccaaggcaccactctcacagt
ctcctcagccaaaacaacacccc

Figure 9b

<u>gacgtccagatccagcagcctgggactgagcttgtgaagcctggggcttc</u> PCR primer	50
agtgagactgtcctgcaaggcttctggctacaccttcacc <u>acctactgga</u> CDR I	100
<u>tgcact</u> gggtgaagcagaggcctggacaaggccttgagtggatcgga <u>gag</u> CDR II	150
<u>attgatccttctgatagttataactataatcaaaagttcaagggc</u> aa	200
ggccacattgactgtagaca ^{aa} tctccagcacagcctacatgcacctca	250
gcagcctgacatctgaggactctgcggtctattactgttcaaga <u>aactac</u> CDR III	300
<u>ggtagtggctactactttgactact</u> tggggccaaggcaccactotcacagt	350
ctcctcagc ccaaaacaacacccc constant region	373

Figure 10 9D33 Heavy Chain amino acid sequence

Figure 10a

DVQIQQPGTELVKPGASVRLSCKASGYTFTTYWMHWVKQRPGQGLEWIGE

IDPSDSYTNYNQKFKGKATLTVDKSSSTAYMHLSSLTSEDSAVYYCSRNY

GSGYYFDYWGQGTTLTVSSAKTTP

Figure 10b

DVQIQQPGTELVKPGASVRLSCKASGYTFT	TYWMH	WVKQRPGQGLEWIGE	50
PCR primer	CDR I		
IDPSDSYTNYNQKFKG	KATLTVDKSSSTAYMHLSSLTSEDSAVYYCSR	NY	100
CDR II		CDR III	
GSGYYFDY	WGQGTTLTVSS	AKTTP	124
	constant region		

Figure 11 9D33 Light Chain nucleotide sequence

Figure 11a

ggcgttgagatgacacagtcgccagcaatcatgtctgcatctccagggga
 gaaggtcaccatgacctgcagtgccagctcaagtgtaagttacatgcact
 ggtaccagcagaagtcaggcacctccccaaaagatggatttatgacaca
 tccaaactggcttctggagtcctgctcgcttcagtggcagtgggctctgg
 gacctcttactctctcacaatcagcagcatggagactgaagatgctgcca
 cttattactgccagcagtgagtagtaaccgctggacgcttcggtggaggc
 accaaactggaaatcaaacggctgatgctgc

Figure 11b

ggcgttgagatgacacagtcgccagcaatcatgtctgcatctccagggga	50
PCR primer	
gaaggtcaccatgacctgcagtgccagctcaagtgtaagttacatgcact	100
CDR I	
ggtaccagcagaagtcaggcacctccccaaaagatggatttatgacaca	150
CDR II	
tccaaactggcttctggagtcctgctcgcttcagtggcagtgggctctgg	200
gacctcttactctctcacaatcagcagcatggagactgaagatgctgcca	250
CDR III	
cttattactgcagcagtgagtagtaaccgctggacgcttcggtggaggc	300
accaaactggaaatcaaacggctgatgctgc	331
constant region	

Figure 12 9D33 Light Chain amino acid sequence

Figure 12a

GVEMTQSPAIMASAPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDT

SKLASGVPARFSGSGSGTSYSLTISSMETEDAATYYCQWSSNPWTFGGG

TKLEIKRLML

Figure 12b

GVEMTQSPAIMASAPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDT	50
PCR primer CDR I	
SKLASGVPARFSGSGSGTSYSLTISSMETEDAATYYCQWSSNPWTFGGG	100
CDR II CDR III	
TKLEIKRLML	110
constant region	